ORIGINAL ARTICLE



Acute LED irradiation does not change the anaerobic capacity and time to exhaustion during a high-intensity running effort: a double-blind, crossover, and placebo-controlled study

Effects of LED irradiation on anaerobic capacity and performance in running

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Abstract The purpose of this study was to investigate the acute effects of photobiomodulation therapy using cluster light-emitting diodes (LEDT; 104 diodes) (wavelength 660 and 850 nm; energy density 1.5 and 4.5 J/cm²; energy 60 J at each point; total energy delivered 600 J) on alternative maximal accumulated oxygen deficit (MAOD_{ALT}) and time to exhaustion, during a high-intensity running effort. Fifteen moderately active and healthy males (age 25.1 ± 4.4 years) underwent a graded exercise test and two supramaximal exhaustive efforts at 115 % of the intensity associated with maximal oxygen uptake performed after acute LEDT or placebo irradiation in a double-blind, crossover, and placebocontrolled study design. The MAODALT was assumed as the sum of both oxygen equivalents estimated from the glycolytic and phosphagen metabolism pathways during each supramaximal effort. For the statistical analysis, a paired t test was used to determine differences between the treatments. The significance level was assumed as 95 %. In addition, a qualitative analysis was used to determine the magnitude of differences between groups. No significant differences were found for the values of oxygen equivalents from each energetic metabolism ($P \ge 0.28$), for MAOD_{ALT} values between the LEDT

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and placebo conditions ($P \ge 0.27$), or for time to exhaustion (P = 0.80), except for the respiratory exchange ratio (P = 0.01). The magnitude-based inference of effect size reported only a possibly negative effect of photobiomodulation on MAOD_{ALT} when expressed in units relative to body mass and on the glycolysis pathway (26 %). In summary, LEDT after a high-intensity running effort did not alter the MAOD_{ALT}, metabolic energy pathways, or high-intensity running performance.

Keywords Anaerobic capacity \cdot LED therapy \cdot Maximal accumulated oxygen deficit \cdot Photobiomodulation therapy

Introduction

Photobiomodulation therapy is a type of light therapy that utilizes non-thermal and non-ionizing light in the visible and infrared spectrum [1]. Its effect has been associated with electron transport chain activity, increasing the oxidative metabolism and adenosine triphosphate (ATP) production [2], causing changes in the oxygen kinetics [3], phosphocreatine resynthesis [4], lactate production [4, 5], and microcirculation [6], contributing to increases in the supply of energetic substrate in the muscle.

In this way, some recent studies have reported improvement in performance after acute photobiomodulation [3, 6, 7]; however, the effects of photobiomodulation therapy on a high-intensity effort are still unclear [8], and based on the increase in ATP production [2], phosphocreatine resynthesis [4], and reduction in blood lactate concentration ([La⁻]) [4, 5], it is possible to assume that photobiomodulation therapy could also improve the high-intensity effort such as through the

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anaerobic capacity and time to exhaustion in a supramaximal effort.

Recently, some studies [9–13] have reported the use of blood lactate responses and the fast phase of the excess postexercise consumption to estimate the maximal accumulated oxygen deficit (i.e., a reliable [12] and most scientifically accepted procedure to assess anaerobic capacity) in a single supramaximal effort (MAOD_{ALT}). However, considering that the MAOD_{ALT} is determined exclusively by the delta of the blood lactate ([Δ La⁻]) and the excess post-exercise consumption [14, 15], it is plausible to assume that photobiomodulation applied before the effort could modify the MAOD_{ALT} and enhance the time to exhaustion and other energetic parameters during a high-intensity effort.

The possibly positive effect of photobiomodulation on the anaerobic variables (i.e., phosphocreatine resynthesis and reduction of blood lactate ([La⁻]) could enable the use of this method to improve performance in efforts where anaerobic capacity is a performance determinant; therefore, our hypothesis was that the photobiomodulation therapy would play an important role in the energy release, improving high-intensity effort performance and consequently altering the MAOD_{ALT}. This way, the purpose of this study was to investigate the acute effects of photobiomodulation therapy using cluster multidiodes (LEDT), on the MAOD_{ALT}, metabolic energy pathways, and time to exhaustion during a high-intensity running effort.

Methods

Participants

The minimum sample size for a statistical power of 90 % (alpha 0.05) was 10 participants. The sample size was calculated based on the De Machi et al. [3] investigation using the time-to-exhaustion data.

In this way, 15 Caucasian males composed by students and professionals of physical education participated in the present study (mean \pm SD; age 25.1 \pm 4.4 years; body mass 73.6 \pm 9.7 kg; height 177.3 ± 7.0 cm; body mass index $23.2 \pm$ 2.2 kg m⁻²; maximal oxygen uptake $50.4 \pm$ $3.9 \text{ mL kg}^{-1} \text{ min}^{-1}$). All participants were instructed to avoid strenuous exercises 48 h prior to the efforts and not to ingest any nutritional supplements during the study. Before starting the procedures, all subjects were informed about the risks and benefits involved in the tests and signed the consent forms. All procedures were approved by Ethics Committee on Research Involving Human Subjects from the Faculty of Sciences Human, UNESP-Univ Estadual Paulista (process number 1.192.274) and were conducted in accordance with the ethical principles for medical research involving human subjects (Declaration of Helsinki, 1964).

Inclusion and exclusion criteria

The inclusion criteria were as follows: moderately active males (weekly physical activity between 100 and 200 min); healthy and without any vascular disease, metabolic disorders, recent muscle-skeletal or joint injuries; non-smoking; and aged between 18 and 35 years. The exclusion criterion is if the participant had the following: the use of regular nutritional ergogenic aid or pharmacological drugs; regular absences at the trials; occurrence of muscle damage or muscle pain during the study; and the ingestion of alcohol, caffeine, or any other substances prior the trials, which can alter the exercise performance and metabolic responses. Female participants were not included in the study in order to homogenize the sample (i.e., males can be an anaerobic capacity 32 % higher than women [16]) and to avoid effects generated by the hormonal imbalance during the menstrual cycle [17].

Experimental design

The study was designed as double-blind, randomized, crossover, and placebo-controlled (i.e., using blindfolds and wearing headphones to avoid perceiving light and sound signals during the LEDT session). All tests were performed on a motorized treadmill (ATL, Inbramed, Inbrasport, Porto Alegre, Brazil) with a fixed gradient of 1 % [18]. Prior to the tests, a warm-up lasting 5 min was performed at 8 km h⁻¹. During the tests, the participants wore a safety belt attached to their chest to ensure maximal effort [10, 11].

The subjects performed three visits to the laboratory and underwent a graded exercise test (GXT and two supramaximal efforts at 115 % of the intensity associated with maximal oxygen uptake [10, 11]. The supramaximal efforts were performed prior to the irradiation with the LEDT or a placebo and in a randomized order [19]. A minimum recovery interval of 48 h was adopted between each exercise session. The experimental design is shown in Fig. 1.

Physiological and metabolic measurements

Blood samples (25 μ L) were collected from the earlobe 3, 5, and 7 min after the GTX, and for the supramaximal effort the blood samples were collected at rest after 10 min of quietly sitting (i.e., before the warm-up or any effort) and at the 3rd, 5th, 7th, and 10th minutes after the effort. The samples were analyzed in an automated electrochemical analyzer (YSI 2300 STAT PLUS, Yellow Springs Instruments, Yellow Springs, USA) for determining the [La⁻].

During all tests, the respiratory responses were registered breath by breath using a gas analyzer (Quark PFT, Cosmed, Rome, Italy). In the supramaximal effort test, the oxygen uptake (VO_2) was measured at rest (i.e., 10-min sitting) for baseline values, during the test and 7 min after the end of

Fig. 1 Experimental design



the exercise, for determination of the fast component of excess post-exercise oxygen consumption.

The gas analyzer was calibrated using ambient air and a sample of known gases (5.06 % CO₂ and 16.02 % O₂; White Martins, Osasco, Brazil) and a spirometer with a 3-L syringe (Hans Rudolf, Kansas City, USA), according to the manufacturer's recommendations. For analysis of respiratory variables, the data were smoothed every five points and interpolated every 1 s using the software OriginPro 8.0 (OriginLab Corporation, Northampton, USA). Heart rate was measured during all efforts using a transmitter belt coupled to the gas analyzer (wireless HR 138 monitor, Cosmed, Rome, Italy).

In all tests, the rate of perceived exertion (RPE) was measured immediately after the end of the efforts using the 6–20 Borg scale [20]. During the GXT, the RPE was also measured immediately after each exercise stage.

Graded exercise test

The GXT was performed to determine the maximal oxygen uptake (VO_{2MAX}) and minimal exercise intensity at which VO_{2MAX} was reached (iVO_{2MAX}). The test started at 8 km h⁻¹, and the exercise velocity was increased by 1.5 km h⁻¹ every 2 min until voluntary exhaustion, given voluntarily by the participant or by the inability to perform the effort at the pre-determined speed. The protocol was selected in view of the optimal test duration (8– 10 min) [21].

For the iVO_{2MAX} determination, the VO₂ mean of the final 30 s of each stage was measured. If the plateau in oxygen consumption (range <2.1 mL kg⁻¹ min⁻¹) in the final two stages was not reached, the following criteria were considered to assume VO_{2MAX}: (1) respiratory exchange ratio >1.10; (2) maximum heart rate>90 % of maximum predicted heart rate (220 years of age); and (3) peak blood lactate concentration ([La⁻_P]) \geq 8.0 mmol L⁻¹ [22].

Supramaximal efforts

Initially, the participants remained sitting for 10 min for measurement of the baseline values of oxygen uptake (VO₂) and blood lactate ($[La_B]$). The subjects performed two identical supramaximal efforts at 115 % of the iVO_{2MAX} assessed in the GXT [12]. The time to exhaustion was recorded.

MAOD_{ALT} determination

The MAOD_{ALT} was determined using the method suggested by Bertuzzi et al. [9], based on the sum of the oxygen equivalents from the glycolytic ($W_{[La_{-}]}$) and phosphagen (W_{PCR}) pathways. The $W_{[La_{-}]}$ was estimated using the accumulated [La_] during the exercise (i.e., $\Delta[La_{-}] = [La_{-}P] - [La_{-}B]$), assuming an equivalent of 3 mL kg⁻¹ of oxygen per each 1 mmol L⁻¹ of the $\Delta[La_{-}]$ [9–12]. The W_{PCR} was estimated using the fast phase of excess post-exercise oxygen consumption calculated by a bi-exponential mathematical fit (Eq. 1) [9–13, 15]

$$\operatorname{VO}_{2(t)} = \operatorname{VO}_{2 \operatorname{BASE}} + A_1 \left[e^{-\frac{t\cdot\delta}{\tau_1}} \right] + A_2 \left[e^{-\frac{t\cdot\delta}{\tau_2}} \right]$$
(1)

in which $VO_{2(t)}$ is the oxygen uptake at time t, VO_{2BASE} is the oxygen uptake at baseline, "A" is the amplitude, " δ " is the time delay, and " τ " is the time constant. The "1" and "2" represent the fast and slow components, respectively, and the fast phase of excess post-exercise oxygen consumption was calculated by the product of "A1" and " τ 1".

LED therapy

The LEDT (THOR-LX2, Thor Photomedicine Ltd, London, UK) was performed using a cluster multi-diode containing 104 LEDs. The sessions were conducted by a technician who preset the control unit in the on- and offmodes (i.e., on-mode: LEDT; off-mode: placebo), accordingly to treatment mode. In addition, to ensure that the

Table 1 Parameters for the LED therapy application

Parameters	Specifications
Number of LEDs	104 (56 diodes of 660 nm and 48 diodes of 850 nm)
Wavelength	Mixed, of 660 and 850 nm
Frequency	0–1500 Hz
Optical output	10 mW (660 nm) and 30 mW (850 nm)
LED spot size	69 cm^2
Power density	$50 \ \text{mW/cm}^2$ (660 nm) and 150 mW/cm^2 (850 nm)
Energy	60 J at each point (0.3 J from each red LED and 0.9 J from each infrared LED)
Energy density	1.5 J/cm ² from each red LED and 4.5 J/cm ² from each infrared LED
Total energy delivered	600 J (300 J per leg)
Treatment time	30 s at each point

double-blind design was kept, the technician did not perform any procedure during the exercise sessions and data/ statistical analysis. The technician was instructed to communicate the type of treatment neither to the participants nor to the researchers. The participants were blindfolded and wore headphones (i.e., hearing a standard song) so that the light and sound signals were not perceivable [19]. The order of the treatments was determined by means of a simple randomization method (raffle) [23].

The LEDT protocol had an overall duration of 2.5 min, 30 s per point, with application in both legs simultaneously. The application was performed in two regions of the quadriceps muscle, two regions of the biceps femoris, and one region between the soleus and gastrocnemius muscles following the distribution axis of the muscle fibers in both legs. The interventions were performed using the Spot method with direct contact (90° angle) of the equipment on the skin surface. The technical and procedural parameters are shown in Table 1.

Fig. 2 Comparison between the $MAOD_{ALT}$ values in the LEDT and placebo conditions expressed in absolute (a) and relative to body mass (b) units

Statistical analysis

For the statistical analysis, the software SPSS Statistics 20.0 (IBM, Germany) was used. Initially, to determine the normal distribution of the data, the Kolmogorov-Smirnov test was applied and the results demonstrated a normal Gaussian distribution. A paired t test was used in all parameters to examine differences between the LEDT and placebo conditions. The Pearson's correlation test was applied, to determine the associations between the MAOD_{ALT} (MAOD_{LED} and MAOD_{PLA}) and the metabolic contributions; to verify the associations between the MAOD_{ALT} and performance values; and to verify possible associations between the variables. The statistical power of the MAOD_{ALT}, metabolic parameters, and performance values was determined using the software G*Power (3.0.10, Heinrich-Heine University, Düsseldorf, Germany). The significance level was assumed as 95 % in all cases (P < 0.05).

In addition, as an additional qualitative analysis, the magnitude of differences between groups was calculated and expressed as standardized mean differences (Cohen's *d*) [24]. The threshold values for the Cohen's *d* statistics were >0.2 (small), >0.5 (moderate), and >0.8 (large). The chances of a possible substantial benefit or harm were calculated (0.2 multiplied by the between-subject deviation). The changes were qualitatively evaluated as follows: <1 % = most unlikely; 1-5 % = very unlikely; 5-25 % = unlikely; 25-75 % = possibly; 75-95 % = likely; 95-99 % = very likely; and >99 % = most likely. When the positive and negative values were both >5 %, the inference was classified as unclear.

Results

All subjects achieved the exhaustion criteria during the GTX, and the physiological parameters were assumed as maximal. The test lasted 11.1 ± 1.7 min, and the iVO_{2MAX} achieved was





 14.6 ± 1.3 km h⁻¹. The exhaustion values obtained in the test were 50.4 ± 3.9 mL kg⁻¹ min⁻¹ for the VO_{2MAX}, 1.18 ± 0.09 for the respiratory exchange ratio, 192.1 ± 10.8 beats min⁻¹ for the heart rate, and 11.2 ± 2.6 mmol L⁻¹ for post-exercise [La⁻_P]. The RPE after the test was 18.7 ± 1.4 .

The values of MAOD_{ALT} at 115 % of iVO_{2MAX} (16.8 ± 1.5 km h^{-1}) are shown in Fig. 2. The MAOD_{ALT} in the LEDT and placebo conditions did not differ when expressed in absolute units (*P*=0.27; *statistical power* 0.54) or relative to body mass (*P*=0.29; *statistical power* 0.70). However, the magnitude-based inference of effect size reported a possibly negative effect of the LEDT on the MAOD_{ALT} when expressed in units relative to body mass (42 %) (Fig. 3). Furthermore, moderate and significant correlations were verified between the MAOD_{ALT} values when expressed in absolute units (*r*=0.74) and relative to body mass (*r*=0.79).

The W_[La⁻], W_{PCR}, and W_{AER} contributions in the supramaximal efforts were 23.5 ± 4.3 , 15.4 ± 3.1 , and $61.1 \pm 5.9\%$ (LEDT condition) and 23.9 ± 4.0 , 15.8 ± 4.2 , and $60.3 \pm 5.4\%$ (placebo condition), respectively. The values of oxygen equivalents from each energetic metabolism during the supramaximal efforts are shown in Table 1. There was no

significant difference between the oxygen metabolic equivalents in the LEDT and placebo conditions. The magnitudebased inference of effect size reported only a possibly negative effect (26 %) of the LEDT on the $W_{[La_{-}]}$, when expressed in units relative to body mass (Fig. 3).

The RPE and exhaustion parameters after the supramaximal efforts were not significantly different (P > 0.05) in the LEDT and placebo conditions, except the respiratory exchange ratio (Table 2). Furthermore, the VO₂ reached in the supramaximal effort in the LEDT and placebo conditions (48.8 ± 3.9 and 48.4 ± 3.8 mL kg⁻¹ min⁻¹, respectively) was lower than that achieved in the GTX (50.4 ± 3.9 mL kg⁻¹ min⁻¹) (P = 0.01 for both; *statistical power* 0.63 and 0.62, respectively). The magnitude-based inference of effect size reported a possibly negative effect of the LEDT on the [La⁻_P] (28 %) and on the [La⁻] at the 7th (29 %) and 10th (60 %) minutes after the supramaximal effort. In addition, the magnitude-based inference of effect size also reported a possibly negative effect of the LEDT on the area under the curve for the lactate kinetics (50 %).

There was no significant difference (P=0.80; *statistical* power 0.86) between the time to exhaustion in the

Table 2 Difference between the
physiological parameters to
exhaustion and the RPE
immediately after the
supramaximal efforts

	LEDT	Placebo	P value	Statistical power
VO_{2EX} (mL kg ⁻¹ min ⁻¹)	48.8 ± 3.9	48.4 ± 3.8	0.32	0.70
RER _{EX}	1.37 ± 0.09	1.32 ± 0.07	0.01	0.62
HR _{EX} (beats min ⁻¹)	182.7 ± 16.0	182.1 ± 12.0	0.87	0.89
$[La_P] \pmod{L^{-1}}$	12.6 ± 2.1	13.0 ± 2.6	0.34	0.76
RPE _{EX}	18.5 ± 1.5	18.1 ± 1.9	0.24	0.77

Data are reported as mean \pm standard deviation

 VO_{2EX} exhaustion oxygen consumption, RER_{EX} exhaustion respiratory exchange ratio, HR_{EX} exhaustion heart rate, $[La_{P}]$ peak lactate concentration after exercise, RPE_{EX} exhaustion perceived exertion



Fig. 4 Lactate kinetics at 0 (rest), 3, 5, 7, and 10 min after the supramaximal efforts

supramaximal efforts in the LEDT (154.6 ± 36.0 s) and placebo conditions (155.5 ± 37.0 s). Furthermore, a high and significant correlation (r = 0.93) and lower percentage variation (-0.05 ± 8.37 %) were verified between the supramaximal times to exhaustion.

Discussion

The purpose of the present study was to investigate the acute effects of LEDT on the MAOD_{ALT}, metabolic energy pathways, and time to exhaustion during a high-intensity running effort. The main findings were the non-statistical effect of the LEDT on the MAOD_{ALT}, W_{ILa} , W_{PCR} , W_{AER} , and performance (time to exhaustion). Therefore, the initial hypothesis was refuted.

The MAOD_{ALT} suggested by Bertuzzi et al. [9] and recently validated by Zagatto et al. [12] has been used to evaluate the effects of ergogenic substances on performance and the metabolic pathways [10, 11]. In the MAOD_{ALT} protocol, the $W_{[La^-]}$ is determined by the $\Delta[La^-]$ and the oxygen equivalent [9–13]. Therefore, alterations in the [La⁻] may change the $W_{[La^-]}$ during the test. In the present study, only a possibly negative effect of LEDT on the Δ [La⁻], [La⁻_P], and [La⁻] was observed at the 7th and 10th minutes and the area under the curve for the lactate kinetics. Furthermore, a decreasing tendency in the [La⁻] at the 10th minute after the high-intensity running effort in the LEDT condition (Fig. 4) was observed, with a percentage of variation of -4.86 ± 9.90 %.

Reis et al. [5] observed a decrease in [La⁻] at the 10th minute after an intense effort preceded or followed by low-level laser therapy (LLLT) (wavelength 830 nm; energy density 214.28 J/cm²). Leal-Junior et al. [25] also verified changes in the lactate kinetics when LEDT (wavelength 660/850 nm; energy density 1.5/4.5 J/cm²) and LLLT (810 nm and 164.84 J/cm²) were applied before a high-intensity interval training session. A possible explanation for this lactate kinetics may be the photobiomodulation effects on the oxidative metabolism activity, which stimulates the oxidation of the lactate to pyruvate for reutilization in the mitochondria, resulting in decreased values of lactate [4, 5].

In the present study, the W_{AER} , W_{PCR} , and $EPOC_{FAST}$ components ("A₁" and " τ_1 ") were not altered by the LEDT (Table 3). Some studies [3, 26] have verified changes in the oxidative parameters after photobiomodulation therapy sessions. Considering that the oxidative metabolism is primarily responsible for phosphocreatine resynthesis, alterations in mitochondrial activity could alter the oxygen kinetics and the efficiency of phosphocreatine resynthesis and, consequently, change the W_{AER} and the W_{PCR} EPOC components ("A₁" and " τ_1 ") [2]. The results of the present study can be explained by the short duration of the effort (154.6 ± 36.0 and 155.5 ± 37.0 s in the LEDT and placebo conditions, respectively), where at exhaustion, the subjects did not reach the VO_{2MAX} estimated in the GTX, and therefore, the maximal oxidative potential was not reached during the supramaximal effort.

The LEDT did not alter performance during the supramaximal efforts at 115 % of iVO_{2MAX} (P = 0.80). Furthermore, a high and significant correlation (r = 0.93) and a lower percentage of individual variation were found (-0.05 ± 8.37 %). Recently, some studies have reported performance

	LEDT	Placebo	$\Delta\%$	P value	Statistical power
$W_{[La]}(L)$	2.5 ± 0.6	2.6 ± 0.7	-1.41	0.28	0.78
$W_{[La]}^{-}$ (mL kg ⁻¹)	34.4 ± 6.4	35.4 ± 8.0	-1.41	0.33	0.70
$W_{PCR}(L)$	1.7 ± 0.4	1.7 ± 0.4	-0.11	0.76	0,76
$W_{PCR} (mL kg^{-1})$	22.4 ± 4.1	22.8 ± 4.4	-0.11	0.71	0.82
$W_{AER}(L)$	6.9 ± 2.6	6.8 ± 2.6	1.55	0.75	0.81
$W_{AER} (mL kg^{-1})$	93.0 ± 30.4	92.1 ± 29.5	1.55	0.73	0.76
$A_1 (mL \text{ kg min}^{-1})$	19.9 ± 2.2	19.9 ± 4.2	5.87	0.97	0.97
τ_1 (min)	1.1 ± 0.2	1.2 ± 0.3	-0.70	0.54	0.88

Data are reported as mean \pm standard deviation

LEDT LED therapy, W_{ILa} glycolytic oxygen equivalent, W_{PCR} phosphagen oxygen equivalent, W_{AER} oxidative oxygen equivalent, A_I fast component amplitude, τ_I fast component time constant

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improvement after photobiomodulation therapy. De Marchi et al. [3] reported a time-to-exhaustion improvement in a progressive test after an LLLT session (wavelength 810 nm; energy density 164.8 J/cm²). Leal-junior et al. [27] found an improvement in the number of maximal contractions in a strength exercise (wavelength 655 nm; energy density 500 J/ cm²). Zagatto et al. [8] recently verified a moderate effect of LLLT (wavelength 810 nm; energy density 107.1 J/cm²) on the crossbar jump test in water polo players.

Although some studies have verified a positive effect of photobiomodulation therapy on performance, further investigations are necessary to test its effects, mainly to determine the optimal treatment parameters, such as the energy dose. In the present study, the irradiation was 60 J per point (cluster), totalizing 600 J; this energy dose is higher than that used by De Marchi et al. [3] (30 J per site; total 360 J), Zagatto et al. [8] (3 J per point; total 48 J), and Leal-junior et al. [27] (5 J per point; total 20 J). Leal-Junior et al. [28] suggested a dose near to 0.3 to 41.7 J per point or site, since positive effects on performance were obtained using this dose in previous studies [3, 8, 25]. However, it is noteworthy that no studies have investigated the dose limit to obtain improvements in performance. Therefore, the effects of different doses on performance are still unclear.

In the present study, the magnitude-based inference of effect size reported only a possibly negative effect of LEDT on MAOD_{ALT} and $W_{[La_{1}]}$ (when expressed in relative units) with a lower chance percentage and higher chance percentage concentered in the trivial effect (Fig. 3). In view of this, the possible effect of the LEDT was assumed as insignificant in this exercise mode (short-term). In summary, an acute LEDT before a high-intensity running effort at 115 % of iVO_{2MAX} seems not to alter the MAOD_{ALT}, metabolic energy pathways, or high-intensity running.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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